ABSTRACT OF THE INVENTION

A novel co-culture system using human brain endothelial cells (HUBEC) which promotes the expansion of human $CD34^+CD38^-$ cells consistent with the PMVEC system is disclosed. HUBEC were isolated from cadaveric donors, passed in primary culture, cloned and found to be Von Willebrand Factor positive. Cultivation of purified bone marrow $CD34^+$ cells on HUBEC monolayers supplemented with GM-CSF + IL-3 + IL-6 + SCF + flt-3 ligand caused a 14.5-fold increase in total cells, an 6.6-fold increase in $CD34^+$ cells, and, most remarkably, a 440-fold increase in $CD34^+CD38^-$ cells after 7 days. Further, CFU-GM production increased 15.1-fold, BFU-E increased 8-fold, and CFU-Mix increased 5.2-fold. Optimal generation was dependent upon the continued presence of exogenous supplied cytokines. In comparison, identically treated stroma-free suspension cultures supported a 10.2-fold expansion of total cells, a 3-fold increase in $CD34^+$ cells and maintained the $CD34^+CD38^-$ cell pool after 7 days of culture. Moreover, we found that non-brain human endothelial cells isolated from the same donors supported neither the expansion nor the maintenance of human $CD34^+CD38^-$ cells. Although few steady state $CD34^+CD38^-$ cells give rise to visible colony-forming cells in methylcellulose cultures, our FACS based cell cycle and sorting experiments demonstrated the activation of a highly clonogenic $CD34^+CD38^-$ population (24% cloning efficiency) during ex-vivo culture on cytokine treated HUBEC. These results suggest that bone marrow $CD34^+CD38^-$ cells require a stromal cell microenvironment for optimal expansion and that ex-vivo expanded $CD34^+CD38^-$ cells generated in the HUBEC culture system appear to retain some degree of primitive "stemness".